COPY

"Express Mail" mailing number: EV 841 131 452 US
Date of Deposit August 16, 2005
I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450

Christi A. Butner

Christi A. Butner

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Ehringer et al.

Group Art Unit: 1615

Serial No.: 10/627,195

Examiner: Kishore, Gollamudi S

Filed: July 25, 2003

Docket No.: 1577/2/2/2 CON

Confirmation No.: 2707

For: WOUND HEALING COMPOSITIONS AND METHODS OF USE

* * * * * * * * * * * * *

DECLARATION OF WILLIAM D. EHRINGER PURSUANT TO 37 C.F.R. §1.132

Commissioner of Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. My name is William D. Ehringer, Ph.D. and I am a co-inventor of the subject matter disclosed and claimed in the above-referenced U.S. patent application serial no. 10/627,195. I have personal knowledge of all facts stated herein unless otherwise noted.

2. A true and accurate copy of my *curriculum vitae*, which evidences my expertise and credentials, is attached herewith and labeled **Exhibit B**.

3. I have reviewed the Official Action issued by the U.S. Patent and Trademark Office on February 16, 2005 with respect to the above-referenced U.S. patent application. I have also reviewed the references cited therein.

4. The present subject matter makes use of the discovery that lipid vesicles having formulations as disclosed and claimed in the above-referenced U.S. patent application are fusogenic with cellular bi-lipid membranes and can encapsulate ATP and deliver the ATP directly to cells in quantities sufficient to meet cellular metabolic demands, particularly when cells are stressed.

5. These vesicles as presently claimed encapsulate ATP at selected concentrations (e.g., 1 mM to 50 mM) and comprise a phospholipid which is a stable vesicle former (e.g., a phosphatidylcholine) and at least one unstable vesicle forming member (e.g., a polar lipid or PEG) at a ratio of stable vesicle former to unstable vesicle former of 1:1 to 500:1, wherein the vesicle has a fusion rate of at least 20 vesicle fusions/second. Experiments with formulations falling within these ranges have been performed, and true and accurate presentations of the results of these experiments are provided in attached **Exhibit A**.

6. Figure 1 of **Exhibit A** demonstrates that the fusogenic vesicles disclosed in the above-referenced U.S. patent application can deliver ATP to cells in culture at rates sufficient to provide for the metabolic needs of the cells during hypoxic phases. "Vitasol" as referred to in this and all figures of **Exhibit A** refers to

the fusogenic vesicle formulations disclosed and claimed in the above-referenced U.S. patent application. The left graph shows both control and VitaSol vesicle-treated cells are viable during conditions of normoxia. However, after six (6) hours of hypoxia (0.2% oxygen), nearly all of the control cells have perished, whereas the vast majority of VitaSol vesicle-treated cells are still viable. The right graph shows similar results using a model of hypoxia based on treatment of cells with potassium cyanide.

7. Figure 2 again demonstrates that cell viability is maintained during a hypoxic state by administration of VitaSol, but not in control tissues. Figure 2, left graph, further shows that neither an empty vesicle nor ATP delivered without a vesicle is sufficient to maintain cell viability during anoxia. Figure 2, right graph, shows that the concentration of ATP within the vesicle plays a role in determining cell viability during anoxia, with low concentrations resulting in poor viability. We have also determined that high concentrations of ATP in fusogenic vesicles can be detrimental to cells as well. Since the amount of ATP is highly regulated inside of cells, we have demonstrated that excess delivery of ATP can result in cell death.

8. Figures 3-5 together demonstrate the surprising capabilities of the present fusogenic liposomes to supplement cellular metabolic needs when cells are severely metabolically stressed. These experiments show the fusogenic liposomes of the above-referenced U.S. patent application can meet cellular needs for ATP sufficiently to preserve limbs and organs for periods of time far beyond any previously known approach. Figures 3 and 4 demonstrate that a limb severed from a rodent and kept at <u>room temperature</u> in a solution comprising VitaSol for 21 hours can be

reattached to the rodent and remain viable. Figure 3 shows pictures of the VitaSol perfused limb and the control limb (perfused in Eurocollins (EC), a commonly used organ preservation solution) several days post-reattachment. The control limb is necrotic and dark, whereas the VitaSol limb is well-perfused and functional. Figure 4 is a graph showing limb survival. Only VitaSol perfused limbs (circle and star on graph) survived for an extended period of time after reattachment. Figure 5 shows similar results to the limb studies in a model of liver preservation. The left graph shows that 6 of 7 livers stored in VitaSol for twenty (20) hours were viable, whereas only two (2) of 12 livers were viable (but likely damaged as exhibited by increased ALT) when stored in solutions not containing VitaSol. The right graph shows hepatic ATP content decreased significantly less in VitaSol-treated livers as compared to controls not treated with VitaSol.

9. Figure 6 is a series of photographs over time showing skin wound healing rates of mice. Wounds treated with the present fusogenic vesicles encapsulating ATP healed significantly more quickly than wounds of control animals. It can be seen from Figure 6 that by Day 5 of the study the wound treated with the present fusogenic vesicles was much farther along toward healing than the control, and by Day 10, the wound treated with the novel fusogenic vesicles was nearly completely healed. In contrast, the control animal wound was still not completely healed by Day 19. These data again show the surprising effectiveness of the present vesicles to provide needed ATP to stressed tissues, which in this case allowed for the tissue to heal much more rapidly than controls.

10. Figure 7 is a graph showing test animal survival rates after experimentally induced hemorrhagic shock. In these experiments, 33% of circulating blood was removed from experimental and control animals. Experimental animals then received an intraperitoneal injection of VitaSol and control animals received an intraperitoneal injection of saline. Hemodynamics and survival were then measured over time. As the graph in Figure 7 shows, 100% of the VitaSol animals survived for more than 100 minutes and about 70% of the VitaSol animals survived for at least 250 minutes after shock was induced. In contrast less than 20% of the control animals survived past 100 minutes and all of the control animals expired in less than 200 minutes. Again, these data show that the fusogenic liposomes of the above-referenced U.S. patent application can deliver ATP at a sufficient rate to cells to meet the metabolic demands of stressed tissues, surprisingly, even under extreme trauma conditions when, for example, 1/3 of a subject's blood volume is depleted.

11. As evidenced by the data presented above, the fusogenic liposomes encapsulating ATP of the above-referenced U.S. patent application are capable of delivering sufficient ATP to stressed cells to meet the metabolic demands of the cells and thereby increase survival of tissue, even under extreme conditions of cellular stress as occurs during, for example, the diverse conditions of anoxia, limb and organ transplantation, wound healing, and hemorrhagic shock. None of the art of record is believed to provide vesicles with these properties.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true;

Application Serial No.: 10/627,195

and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

William D. Ehringer, Ph.D.

8/16/05

Date